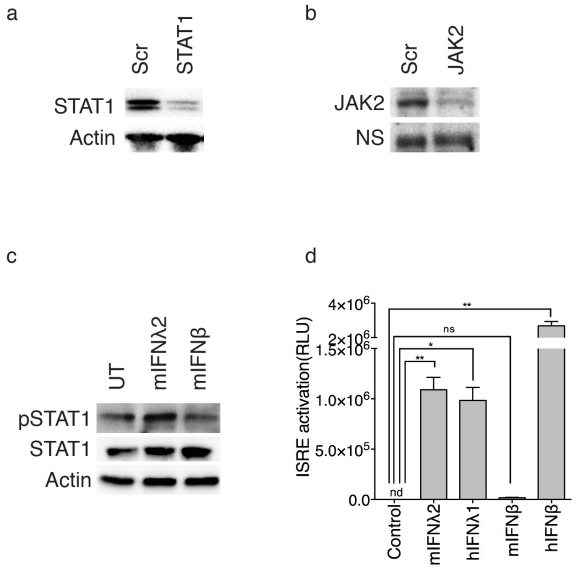
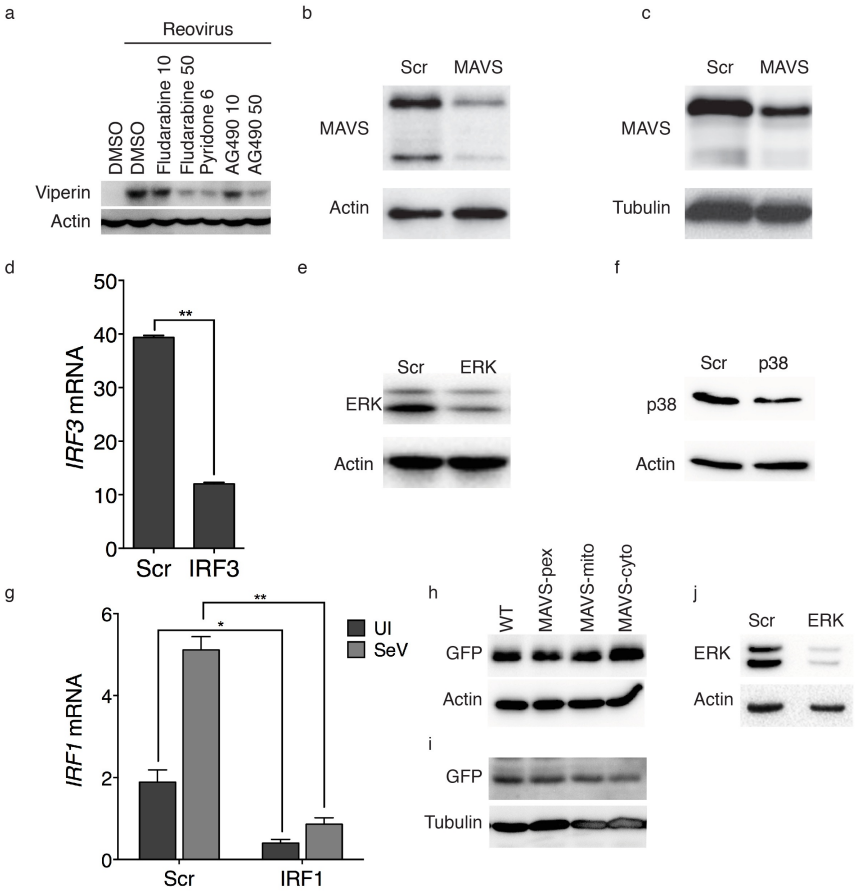
Supplementary Figures and Legends



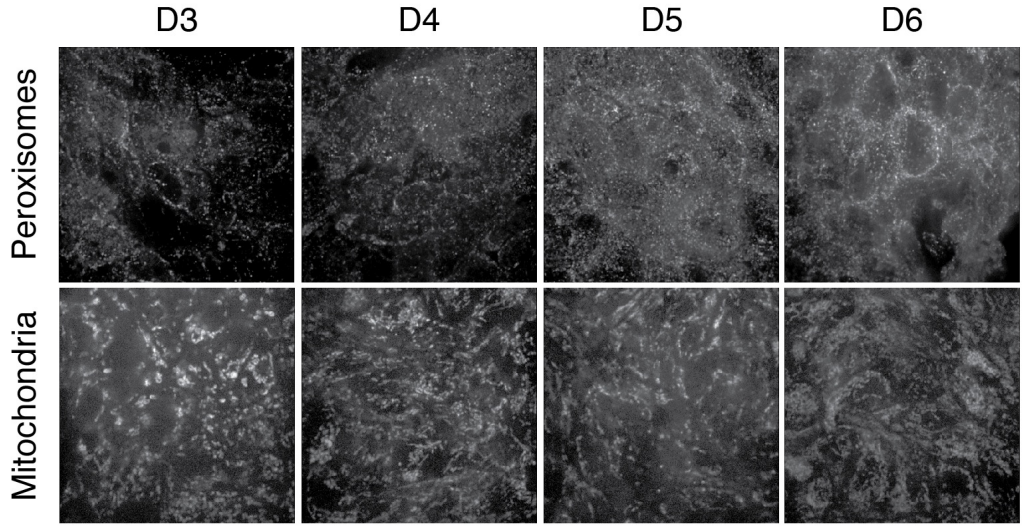
Supplementary Figure 1 : JAK/STAT signaling and the Type III IFN pathway.

(a) STAT1 and (bB) JAK2 were knocked down in Huh7 cells. (c) Human Huh7.5 cells were incubated with mouse IFNλ2 or IFNβ, and STAT1 phosphorylation was assessed by western immunoblotting. (d) 293T cells expressing luciferase under the control of an ISRE promoter were incubated with mouse IFNλ2, human IFNλ1 or mouse or human IFNβ. Cells were then assessed for their ability to respond to IFNs by producing luciferase. Error bars represent mean ± SEM of triplicate readings for one experiment representative of 3. *, $P < 0.01$; **, $P < 0.001$; ***, $P < 0.0001$ (One-way ANOVA).



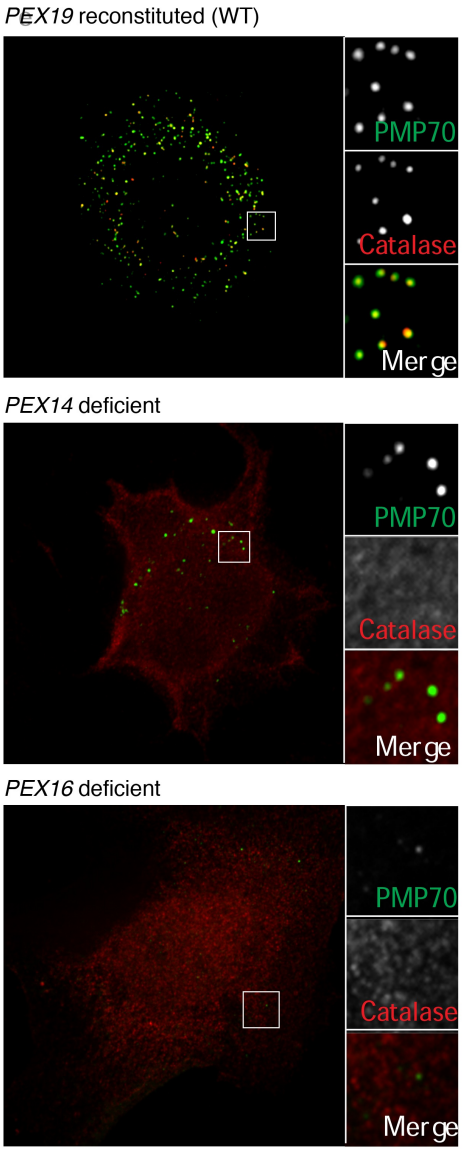
Supplementary Figure 2 : Knockdowns of components of the Type III IFN pathway

(a) Polarized T84 cells were treated with inhibitors Fludarabine (STAT1), Pyridone 6 (JAKs) and AG490 (JAK2). After infection with Reovirus, viperin expression was assessed by western immunoblotting. Huh7 cells (b) or JEG3 trophoblasts (c) were knocked down for MAVS. (d) IRF3 depletion in Huh7 cells. ERK (e) or p38 (f) were knocked down in Huh7 cells. (g) IRF1 was knocked down in Huh7 cells. Cells were infected with SeV, inducing IRF1 in control cells transfected with a scrambled (Scr) siRNA oligo but not in cells transfected with an IRF1 siRNA oligo. (h) Huh7 cells were stably transduced with MAVS chimera localized on peroxisomes (Pex), mitochondria (Mito), both (WT) or neither (Cyto). MAVS transgenes include GFP whose expression is controlled by an IRES. Equivalent transgene expression was assessed by western immunoblotting against GFP. (i) Similar to (h) except JEG3 trophoblasts were transiently transfected with the indicated MAVS transgenes. Equivalent transgene expression was assessed by western blotting against GFP. (j) ERK1/2 was knocked down in MAVS-KO MEFs expressing MAVS-Pex.*, $P < 0.01$;**, $P < 0.0001$ (Student's *t*-test (d), One-way ANOVA (g)). Error bars represent mean ± SEM of triplicate readings for one experiment representative of 3.



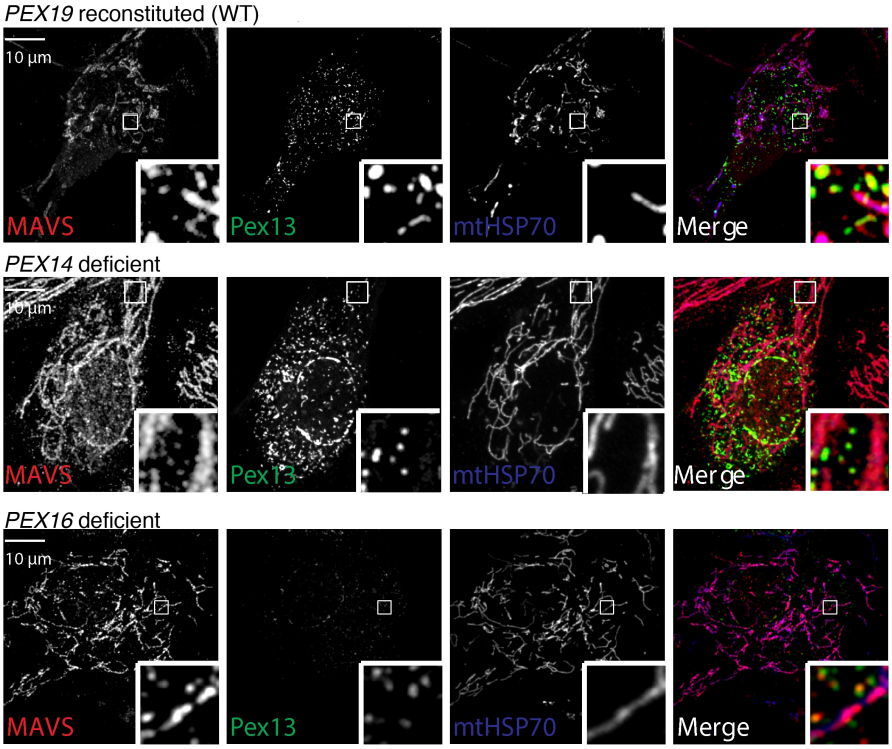
Supplementary Figure 3 : Peroxisome and Mitochondria abundance in T84 cells

T84 epithelial cells were polarized on transwells. Peroxisomes were labeled with Pex14, Mitochondria were visualized with Alexa-conjugated streptavidin that recognizes the high concentration of biotin in mitochondria.



Supplementary Figure 4 : Localization of peroxisomal markers in Zellweger cells

Human skin fibroblasts of indicated genotype were stained for peroxisomes using PMP70 and catalase antibodies.



Supplementary Figure 5 : MAVS localization in Zellweger cells

Human skin fibroblasts of indicated genotype were transfected with *PEX13-GFP* to label peroxisomes. Mitochondria were visualized with anti-mtHSP70 and endogenous MAVS with anti-MAVS.

a

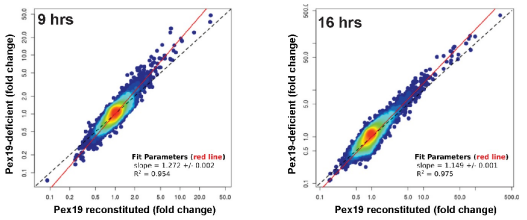
9 hrs post reovirus infection

Pex19 reconstituted			Pex19-deficient		
GO term	GO id	p-value	GO term	GO id	p-value
immune response	GO:0006955	7.62E-16	immune response	GO:0006955	4.68E-21
response to virus	GO:0009615	1.95E-11	response to virus	GO:0009615	1.95E-13
inflammatory response	GO:0006954	1.76E-07	extracellular space	GO:0005615	3.81E-06
chemokine activity	GO:0008009	2.37E-06	inflammatory response	GO:0006954	1.84E-05
extracellular space	GO:0005615	1.36E-04	cell surface receptor linked signal transduction	GO:0007166	7.91E-05

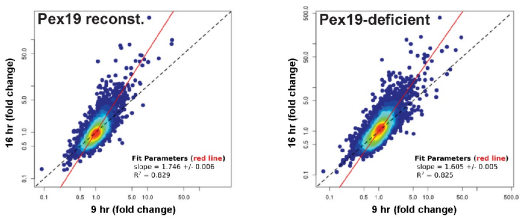
16 hrs post reovirus infection

Pex19 reconstituted			Pex19-deficient		
GO term	GO id	p-value	GO term	GO id	p-value
immune response	GO:0006955	1.05E-36	immune response	GO:0006955	1.37E-38
response to virus	GO:0009615	3.52E-17	extracellular space	GO:0005615	1.29E-19
extracellular space	GO:0005615	8.63E-17	response to virus	GO:0009615	7.24E-16
inflammatory response	GO:0006954	5.37E-14	cell-cell signaling	GO:0007267	3.48E-15
cell-cell signaling	GO:0007267	7.94E-14	chemokine activity	GO:0008009	6.61E-12

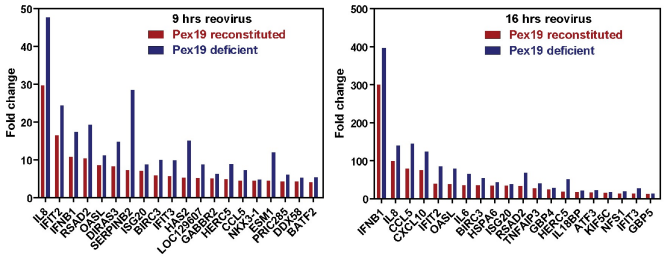
b



c



d



Supplementary Figure 6 : Transcriptome analysis in Zellweger cells

Gene expression profiles of *PEX19*-deficient and reconstituted cells were determined by microarray analysis 9 and 16 hrs after reovirus infection. (a) Table of the most significantly enriched “GO-terms” in regulated genes of *PEX19*-deficient and reconstituted cells.

Pairwise comparisons of both genotypes (b) and time points (c) are depicted in log-log scale scatter plots. The slope of the weighted linear fit indicates stronger gene induction in *PEX19*-deficient cells (B) and the later time point of infection (c) as the fitted slope is significantly greater than 1. (d) Overview of the 20 most highly upregulated genes in *PEX19* reconstituted cells in comparison to *PEX19*-deficient cells.